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CHOLESTEROL CHOLELITHIASIS - ROLE OF THE GALLBLADDER

By

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled:

CHOLESTEROL CHOLELITHIASIS - ROLE OF THE GALLBLADDER

submitted by: RODERICK McDOUGALL

in partial fulfilment of the requirements for the degree of Master of Science.

"Is there any thing whereof it may be said, See, this is new? "

Ecclesiastes 1.10

TO MY PARENTS

ABSTRACT

Cholesterol cholelithiasis is one of the most common surgical illnesses of Western industrialized countries. To date the etiology of the illness is unknown and cholecystectomy is the only accepted form of therapy. Recent reports of the successful dissolution of gallstones by oral administration of chenodeoxycholic acid, a bile acid, hint at a possible alternative to operation. In 1968 Admirand and Small showed that normal bile can be separated from lithogenic or stone-forming bile on the basis of supersaturation with cholesterol. Solubility of cholesterol in bile depends on the production of micelles through the detergent-like action of phospholipids and bile acids, a phenomenon critically related to their relative concentrations. Precipitation of cholesterol from supersaturated bile results in the formation of gallstones. If lithogenic bile is produced indefinitely, medical treatment will have to be lifelong to prevent recurrence, and cholecystectomy becomes an attractive alternative, especially for a young patient.

Bile analysis for phospholipids, bile acids and cholesterol was carried out on patients with normal biliary tracts, gallstones, previous cholecystectomy with, and without, gallstones. Percentage concentrations of these substances were determined and plotted on the triangular coordinates of Admirand and Small, so that the degree of lithogenicity could be determined. It was found that

lithogenic bile persisted indefinitely in patients who had cholecystectomies for cholelithiasis. The present medical therapy for cholelithiasis will therefore require continuous lifelong administration if cholecystectomy is to be ultimately avoided.

By comparing groups it became obvious that the presence or absence of the gallbladder had no significant influence on bile composition. Patients who have normal biliary tracts or who have had cholecystectomies for acalculous disease have normal bile. Subjects with gallstones or who have had cholecystectomies for gallstones have lithogenic bile. The gallbladder therefore plays no role in the formation of lithogenic bile.

A review of the literature suggests that it is dietary factors which produce lithogenic bile. Lack of dietary fibre in industrialized nations affects the enterohepatic circulation of bile salts by increasing their recycling rate and preventing their excretion. The end result is a high incidence of lithogenic bile in industrialized nations, and a rarity of cholesterol gallstones in rural countries.

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INTRODUCTION

The etiological role of the gallbladder in the production of cholesterol gallstones has been speculated about since their discovery. Since it has been shown that precipitation of cholesterol from bile lacking in phospholipids and bile salts produces gallstones, research has focused on isolating those factors which produce a lithogenic bile. Now that it is known that orally administered bile salts result in the dissolution of cholesterol gallstones, it becomes important to know how long a lithogenic bile is produced. Through a study of bile composition before and after cholecystectomy the possible etiologic role of the gallbladder can be elucidated. Furthermore, knowledge of how long lithogenic bile is secreted will allow intelligent selection between current medical therapy and surgery.

LITERATURE REVIEW

Introduction

Extrapolating the statistics reported in the Framingham study to Canada indicates approximately 1.2 million women and 400,000 men have gallstones.[1] The incidence of the 55 - 64 age group is 10% of males and 20% of females.[2] In the United States, surgery is performed eventually in about half of the 800,000 new cases of cholelithiasis each year. 5,000 to 8,000 people die from gallstone disease yearly, and the cost of morbidity approaches 1 billion dollars annually.[3]

Prevalence

World wide prevalence figures indicate that cholesterol cholelithiasis is a disease of industrialized nations. Pure or mixed cholesterol stones (70% or greater by weight) account for 85% of North American gallstones.[4] Pigment stones are the commonest variety in rural Asia and Africa.[5] The Masai tribe of Africa does not have gallstones.[6] Transplanted Africans and Asians have the same incidence as Caucasians.[1] In Japan prior to World War II calcium bilirubinate stones comprised about 70 - 80% of the total. Since the "Americanization" of Japan the incidence of cholesterol gallstones has risen so that only 30 - 40% of stones are the pigment

variety.[7] Calcium bilirubinate stones are found mainly in rural Japan, while cholesterol cholelithiasis appears to be a disease of cities.[8] The North American Indian seems to be an exception to this trend in that a high prevalence of cholelithiasis has been found in several tribes.[9, 10] However, as a general rule, it can be stated that cholesterol cholelithiasis is in some way related to industrialization.

Gallstone Composition

The traditional classification of gallstones was introduced by Aschoff in 1924 and has persisted to date. Stones are inflammatory, metabolic (pure pigment, calcium bilirubinate, or pure cholesterol) combination (primary metabolic plus secondary inflammatory) or stasis (primary in common duct - earthy).[11] Such a classification is now regarded as erroneous. Most calculi are qualitatively similar so any classification which relates composition to etiology is somewhat unjustified.[12] The main constituents of gallstones are cholesterol monohydrate, calcium bilirubinate and calcium carbonate, whether the stones be "pigment" or "cholesterol."[12, 13] Other substances include bile salts, fatty acids, phosphorus, iron, copper, and manganese. Unconjugated bilirubin has been found in the centres of stones.[14] Protein is also a component of gallstones, particularly the centres.[15] Histochemical studies have shown the nucleus or nidus to be a focal collection of mucopolysaccharide. Cholesterol crystals are arranged on a framework of mucopolysaccharide radiating

from the nidus in a concentric manner.[16] It is felt that mucopolysaccharides are important in the formation of gallstones.[7, 17]

Theories of Origin

It has long been held that inflammation and infection are responsible for the formation of gallstones, and explanations as to how infectious agents reach the gallbladder are old and numerous.[18] Naunyn in 1892 proposed that mixed stones arose as a pultaceous mass later invaded by cholesterol. Infection may play some role in the formation of stones either as a primary cause with clumps of dead bacteria acting as a nucleus or nidus, or as a secondary factor.[19] More recently, papillitis, ascending infection and Ascaris infestation have been suggested as causes for the calcium bilirubinate stone in Japan.[8] β-glucuronidase from bacteria deconjugates bilirubin in bile and allows it to form an insoluble precipitate with calcium. Virtually all patients with calcium bilirubinate stones in Japan have been infected with β-glucuronidase producing E. coli.[7] Inflammation from any cause may result in the formation of gallstones. The inflamed gallbladder selectively absorbs bile salts and allows cholesterol to precipitate, bile salts being necessary to maintain cholesterol in solution.[20, 21, 22]

The nucleation theory was introduced by Boysen in 1900. He felt that tiny pigment calculi form in intrahepatic canaliculi as a result of liver disease and migrate to the gallbladder where

cholesterol is added. That bacteria may act as a nucleus has already been mentioned.[19] Mucus plugs, foreign bodies such as suture material, epithelial debris or even parasites may act as nuclei.[23]

In 1757 Coe introduced the concept of stasis as an etiologic factor in gallstone formation. Gallstone formation can be experimentally induced in dogs by incomplete stricture of the common bile duct. Prolonged stagnation allows cholesterol to precipitate from bile.[24] Biliary stasis has been shown to reduce the output of bile acids and lecithin after a short period of time, both of which are necessary for cholesterol solubilization.[25] Gallbladder stasis has been demonstrated during the normal progestational phase of the menstrual cycle, and post-prandial evacuation is delayed after the fourth month of pregnancy. Regular emptying of the gallbladder using cholecystokinin in rabbits on a lithogenic diet does not prevent the formation of gallstones.[26]

Abnormal proteins have been implicated as an etiologic factor. A rather complicated immunochemical study has demonstrated sixteen total biliary proteins and four bile specific proteins. Evidence was also obtained for either or both a missing or abnormal protein in the gallbladder bile of gallstone patients.[27] The absence of a stabilizing lipoprotein has also been investigated.[28]

The endocrine effects of pregnancy and female sex have been related to cholelithiasis.[1, 29] That estrogen tends to make bile

lithogenic and affects bile flow rates has been demonstrated in the primate.[30]

Obesity has also been related to cholelithiasis.[1, 29] Caloric intake is higher in people with gallstones; protein, carbohydrate and lipid composition of the diet is identical to controls. Working time is lower and rest time is significantly higher in women with cholelithiasis. Biliary cholesterol increases when caloric intake is raised.[31] An increased hourly output of cholesterol occurs in a percentage of gallstone patients and this is related to obesity.[32]

Diet may influence the production of gallstones. Increasing daily protein consumption increases the amount of cholesterol produced. Triglyceride and carbohydrate feeding have no effect on bile salt or cholesterol production, while feeding cholesterol increases bile salt synthesis.[31] A high level of dietary cholesterol has been shown to render bile lithogenic.[33] The ability to produce gallstones in hamsters by dietary manipulation has been investigated and confirmed.[34, 35] Protection from the lithogenic diet is obtained by the addition of plant fibre.[36] It is suggested by geographic studies on the incidence of cholelithiasis that lack of dietary fibre may play an important role in the genesis of cholesterol gallstones.[37]

Mucus may well play an important role in the pathogenesis of gallstones. Pathological human bile contains more hexosamine and is more viscous than normal gallbladder bile.[38] It has been

suggested that entrapment of cholesterol crystals by gallbladder mucus allows stone formation to proceed by preventing the normal flushing action of gallbladder contraction from removing them.[26] An increased secretion of mucus precedes the formation of gallstones in rabbits being fed dihydroxycholesterol. This secretion is prevented if pathological bile is not allowed to enter the gallbladder.[39] A prosthesis of mucus is probably essential for the formation of gallstones. Entrapment of cholesterol by mucus, and crystal growth in mucus gels has been demonstrated in Syrian hamsters.[40]

Cholesterol supersaturation of bile is the most accepted theory of gallstone origin and will be reviewed in detail.[41]

The Physicochemical Basis of Gallstone Formation

It has long been known that bile salts have the power to dissolve cholesterol in aqueous solutions, however little research had been done on this prior to the 1930's.[20] In fact the idea that cholesterol precipitation in the gallbladder results from the liver secreting a paucity of bile acids and phosphatides is not new.[42] Early work indicated lecithin, a phospholipid, was a minor constituent of bile. The discovery that lecithin was present in relatively high concentrations promoted much further investigation into the bile salt-lecithin system of cholesterol solubility.[43] By the early 1950's Isaksson showed pathological human bile to be different when the ratio of bile salts plus lecithin to cholesterol was compared to normal.[44] Bile salt-lecithin mixtures were found *in vitro*

to have great dissolving powers for cholesterol, and this verified that lecithin, in combination with bile salts, is indispensable for solubilizing cholesterol in bile.[45]

Detailed investigations of the bile salt-lecithin-cholesterol-water system were completed in the mid-1960's. Initially the ternary system, lecithin-bile salt-water, was studied using X-ray diffraction and microscopic techniques on mixtures of these substances. Information about the physical state of bile, its behaviour and organization into liquid crystals and micelles on the basis of changing concentrations was extrapolated from these studies.[46] Similarly, a second ternary system, lecithin-cholesterol-water, was studied. Cholesterol can be incorporated into the structure of an aqueous lamellar phase by association with lecithin, provided the cholesterol-lecithin ratio is less than one to one. This occurs as a consequence of the side-to-side orientation of the paraffinic parts of these molecules induced by their contact with water.[47] The quaternary system of lecithin-bile salt-cholesterol-water was investigated by the same methods, and the limits for cholesterol solubility defined. The resultant phases, isotropic micellar solution, paracrystalline lamellar, hexagonal and cubic were identified.[48, 49]

Bile salts therefore act like detergents. Beyond a critical concentration in water (critical micellar concentration or CMC) they form polymolecular aggregates termed micelles. Such aggregation can only occur with molecules termed amphipaths: that is, possessing appropriately arranged hydrophobic and hydrophylic regions. Aggregation occurs only above a critical temperature termed the Krafft

point. The structure of a micelle is such that the hydrophylic portions of the component molecules are adjacent to water while the hydrophobic portions are hidden in the centre of the aggregate. Micelles can solubilize non-polar molecules by dissolving them in their hydrocarbon centre. Mixed micelles (micelles formed from more than one component) are created through the aggregation of bile salts and lecithin in water such that a coin-like disc is formed. Bile-salts form the edge, while a double layer of lecithin molecules forms the top and bottom, dissolved cholesterol is interdigitated among the lecithin molecules.[50, 51, 52, 53] A prerequisite for stone formation is oversaturation of bile with cholesterol (i.e., cholesterol concentration outside the zone of micellar solubilization).[48, 49]

A triangular phase diagram for percentage concentrations of cholesterol, phospholipid, and bile salts was derived from these findings and a line of maximum cholesterol solubility drawn. (Fig. 1) A collection of patients with and without gallstones who had bile analyses done were gathered from the literature. When their bile composition was plotted on the triangular coordinates a separation of normal from lithogenic bile on the basis of cholesterol saturation was obtained.[54] In 1968 Admirand and Small collected a series of bile analyses obtained from normal and gallstone containing gallbladders. When the results were plotted on triangular coordinates, pathological human bile was supersaturated with cholesterol when compared to normals.[41]

Many investigators in numerous countries have since confirmed this observation. The geographic incidence of gallstones has been accounted for since low risk populations have less saturated biles than high risk populations. Similarly animals such as the dog, pig, hamster and Rhesus monkey, etc. which do not spontaneously form gallstones, have markedly unsaturated biles.[3, 5, 55, 56, 57, 58]

A few investigators have recently disputed Small's line of maximum cholesterol solubility and proposed new lines which indicate a lesser ability of bile to solubilize cholesterol. It seems likely that the area representing the difference between these lines is a zone of supersaturation in a form of delayed equilibrium, termed metastability.[59, 60, 61,] Although some studies fail to separate normal from abnormal biles,[62, 63] the majority of investigators adhere to the original line of cholesterol solubility and find it provides good clinical separation, which the other solubility lines do not.

Possible reasons for observed clinical discrepancies are numerous. Time of sampling is important since fasting bile is more lithogenic than bile collected at other times.[64] Hepatic bile is also more lithogenic than gallbladder bile.[65, 66, 67] Different methods of handling and analysing bile likely introduce many errors.[68] In a high risk population many so-called normals will be in a pre-stone stage of the disease and have lithogenic bile.[69]

The origin of lithogenic bile appears to be the liver.[66, 67] In fact, it has been proposed that cholelithiasis may be a liver

disease.[70] To understand how bile becomes lithogenic, a review of bile salt metabolism and physiology becomes necessary.

Bile Acid Metabolism and Physiology, the Enterohepatic Circulation

Bile acids are synthesized in the liver from cholesterol by the hepatocyte. These are the primary bile acids, chenodeoxycholic and cholic acids which at physiological pH in the biliary tree and gut exist in the ionized or salt form. Various alterations of the steroid nucleus occur during the synthesis of bile acids. Initially the enzyme 7α-hydroxylase adds a hydroxy radicle, then the hydroxyl group in the three position changes from α to β The 5 double bond of the cholesterol molecule is reduced, and in the case of cholic acid a further hydroxy radicle is added through the action of 12α-hydroxylase before the side chain is shortened by three carbon atoms.[71, 72] This additional hydroxylation step provides the basis for separation of bile acids into dihydroxy (chenodeoxycholic) and trihydroxy (cholic) acids.

The cholanoic acids normally enter bile as glycine or taurine conjugates. Normal glycine to taurine ration is 3:1, but is easily modified by nutritional, hormonal, and other factors.[71] Although much of this work is derived from animal investigations, confirming evidence is accumulating in human studies.[73]

Bile salts undergo an enterohepatic circulation; they enter the gut, are absorbed, return to the liver via the portal vein, are reprocessed and re-excreted.[74] Synthesis of bile salts is apparently

under a negative feedback control at the level of 7α-hydroxylase. New bile acids are produced in response to loss from the system (feces), whereas a sufficient rate of return to the liver from the enterohepatic circulation shuts down synthesis.[75, 76, 77] Normally 200 - 500 mg of new bile acids are produced per day to compensate for fecal loss. Acute interruption of the enterohepatic circulation causes synthesis to increase to maximum rates after several hours, approaching four to five times normal rates in the primate.[72] Man can compensate for a 16% interruption. Cholate synthesis can increase to 2.1 gm per day chenodeoxycholate to 1.2 gm per day, for a total of 3.3 gm per day. Under these conditions the normal cholate to chenodeoxycholate ratio changes from 1.2 to 3.2.[78] There is a critical and somewhat narrow range of return below which no inhibition occurs, and above which no synthesis occurs.[71] It is likely that inhibitory levels are similar for all hepatocytes; however, since hepatocytes take up and secrete bile acids from the portal radicles, there will be a concentration gradient from the portal system to the central vein in any liver lobule. Only when there is sufficient bile salt present to overwhelm the absorptive capacity of hepatocytes near the portal system will the hepatocytes bordering the central vein be inhibited.[74]

Once in the intestines bile salts come under the enzymatic attack of obligate anaerobes such as Bacteroides, are deconjugated and dehydroxylated. Cholic acid becomes deoxycholic acid (dihydroxy) and chenodeoxycholic acid becomes lithocholic acid (monohydroxy).

In addition to these secondary bile acids there are a large number of other bacterial breakdown products including 3β-hydroxy-5β-cholanic, 3α-hydroxy-12-oxo-5β-cholanic, and 3β-12α-dihydroxy-5β-cholanic acid, which are not absorbed. Deconjugation occurs mainly in areas of stasis, the terminal ileum and large bowel. The unique condition of ileostomy patients indicates dehydroxylation occurs in the colon since no secondary bile acids are found in these patients.[79] Interestingly, the secondary bile acid deoxycholate specifically inhibits synthesis of chenodeoxycholic acid but not cholic acid.[80]

Absorption of bile acids occurs throughout most of the gut. After travelling through the biliary system and traversing the duodenum, bile enters the jejunum where some absorption occurs. Most absorption occurs in the distal ileum by an active transport system, dihydroxy salts being absorbed less efficiently than trihydroxy salts; taurine conjugates faster than glycine. Passive absorption also occurs, free acids the fastest, glycine conjugates more slowly, and taurine conjugates hardly at all. Secondary bile acids are mainly formed and absorbed in the colon. Deoxycholate is well absorbed while lithocholate and other products are scarcely absorbed at all.[52, 72] Bile acids are transported via the portal vein to the liver bound to albumen, here they are reconjugated and re-excreted.

The bile salt pool, estimated at between 2 and 4 gms circulates six to ten times per day with intermittent storage in the gallbladder.[81, 82, 83] Gallbladder storage overnight affects bile

composition by interrupting the enterohepatic circulation such that morning fasting hepatic bile has a lower bile salt concentration than at other times during the day.[64] Any factor which interferes with bile salt absorption or kinetics, such as ileal or gallbladder disease or surgery, liver disease, stagnant loop syndrome or diarrhoea to name a few, will alter bile composition.[52, 72, 74, 84] Attention has focused recently on the role of dietary fibre and its effects on bile composition.[37] Plant fibre contains a non-absorbable component, lignin, which adsorbs bile salts in a manner not unlike cholestyramine.[85, 86] Addition of crude fibre to the diet promotes faster intestinal transit and also by its adsorptive properties, enhances bile salt excretion.[87] Such dietary alterations can have profound effects on bile composition, as will be discussed later.

Bile salt secretion influences gastrointestinal and biliary physiology in many ways. Cholesterol metabolism is influenced in several ways by bile salts aside from being their parent compound. Formation of micelles seems to be one of the major determinants of cholesterol excretion by the hepatocyte, although some cholesterol is excreted in the absence of bile salts.[88] The solubilization of cholesterol in bile has been previously discussed.[49] Intestinal micellarization is necessary for digestion and absorption, absence of bile salts results in complete cessation of cholesterol absorption. Dietary cholesterol inhibits synthesis whereas bile acid synthesis induces the liver to manufacture cholesterol. Intestinal synthesis appears to be primarily under the control of

bile acids, being shut off in their presence.[89, 90] It should be noted that a portion of cholesterol undergoes an enterohepatic circulation.[72] Choleresis is stimulated by bile salt flow, and it has been demonstrated that phospholipid secretion is dependent on bile salts in several species.[91] Lecithin accounts for about 98% of the phospholipids in bile.[92] Not only is its secretion dependent on bile salts, synthesis is also claimed to be under their control.[92, 93, 94, 95] Most of the lecithin is destroyed by pancreatic phospholipases in the gut, however an insignificant amount finds its way back to the liver.[96]

<u>Bile Acids in Cholesterol Cholelithiasis; Role of the Gallbladder</u>

As previously discussed a relative lack of liver secretion of bile acids results in a bile supersaturated with cholesterol. It has been shown that gallstone patients have a reduced bile acid pool, and that this reduction in pool size precedes the formation of lithogenic bile.[84, 97, 98, 99, 100, 101] Chenodeoxycholate pool size is particularly reduced in gallstone patients.[97, 102] The secondary bile acid, deoxycholate, comprises a much larger percent of total pool when compared to normals,[99, 101] and the ratio of tri- to dihydroxy acids is changed from 1.23/1 to 0.65/1.[103] In spite of a reduced pool size, synthetic rates are the same as, or only slightly lower than, normal.[97, 101] Cholate and chenodeoxycholate half-lives are much reduced, daily turnover rate being much higher in gallstone patients.[101] A high re-cycling rate is

associated with small pools so that total daily secretion is similar to that seen with larger pools.[104, 105] An inverse relationship between pool size and re-cycling rate has been shown.[106] The breakoff point in pool size between normal and lithogenic bile is estimated to be between 1.6 and 1.8 grams.[107]

To explain cholelithiasis, decreased pool size similar synthetic rates, increased turnover rates, changed bile acid ratios and increased re-cycling must be correlated to the effects of industrialization. These facts can be explained on the basis of colonic stasis and decreased bile salt excretion found in the residents of industrialized countries.[37] Increased stasis from any cause will result in an overproduction of deoxycholate from the dehydroxylation of cholic acid. This causes a reduction in cholate pool size,since there is less cholate for absorption,and deoxycholate specifically suppresses the synthesis of chenodeoxycholate, thus explaining the change in tri- to dihydroxy ratios and the observed decrease in chenodeoxycholate.[80, 102, 103] Pool size will shrink in the presence of stasis. Continual increased resorption will suppress synthesis when stimulus for production of new bile acids should be at its daily maximum, since normally the enterohepatic circulation is interrupted by gallbladder storage during the overnight fast.[64] Recall that it takes several hours for synthetic rates to climb following interruption of the enterohepatic circulation.[72, 108] Turnover rates are increased with a small pool, since daily excretion in the normal range constitutes a

higher percentage of total than if the pool were large. Increased re-cycling is a consequence of the absence of bile salt sequestering agents such as lignin in combination with prolonged opportunity for resorption. Thus the rural African on a high fibre diet rich in lignin averages 400 - 500 gm of stool per day with a transit time of 35 hours, compared to someone on a low fibre diet who averages 150 gm of stool per day with a transit time of over 70 hours.[37] Bile salt excretion is similarly affected.[87] The net result is increased stimulus for synthesis, reduction of deoxycholate and an expanded bile salt pool rich in chenodeoxycholate, and absence of gallstones. That dietary fibre expands the chenodeoxycholate pool and reduces deoxycholate has been demonstrated in man.[109] In addition to this mechanism chenodeoxycholate appears to suppress liver synthesis of cholesterol,[110] and cholesterol output is also related to high caloric intake.[32]

The gallbladder, aside from being a reservoir in which stones can form, affects biliary kinetics by its ability to periodically interrupt the enterohepatic circulation.[64, 84] The effect of cholecystectomy therefore is to remove periodic interruption of the enterohepatic circulation and consequently increase re-cycling rate. This, of course, would reduce the bile salt pool since daily 24-hour output (pool size times re-cycling rate) appears to be rather constant in man.[111] Measured pool size has been found to be reduced[57, 84, 112] although one study indicates pool size returns to normal.[100] Deoxycholate pool is increased post-

cholecystectomy; this is explained by the more constant exposure of bile to intestinal flora.[84, 113] Resultant effects on lithogenicity have been disputed. Several studies show cholecystectomy returns bile to normal[55, 56, 114, 115] while others indicate bile remains lithogenic.[57, 116, 117]

Some researchers feel that a relative lack of bile salt secretion will be seen with reduced pools and will therefore result in a relative depression in phospholipid secretion, and make bile even more lithogenic.[101, 104, 107] This mechanism seems somewhat unlikely in view of the fact that 24-hour output tends to remain constant in man in spite of reduced bile salt pools.[111] It is important to note though that compared to cholic acid, chenodeoxy-cholate causes decreased cholesterol output and increased phospholipid output.[118]

Dissolution of Cholesterol Gallstones

In 1937 Rewbridge reviewed the status of bile acids, cholesterol solubility and gallstones. On the postulate that a bile acid deficiency may be responsible for cholesterol precipitation, oral bile acids were given to five patients. Radiographic disappearance of gallstones was recorded in two of these patients following nine months of treatment.[119] Little was added to this information until the 1970's when the effect of administering pure bile acids to gallstone patients was investigated. That bile salts

administered orally increase the flow of biliary bile salts and phospholipids has been shown.[120] Oral chenodeoxycholic acid, as compared to other bile acids, increases significantly the bile acid plus phospholipid to cholesterol ratios.[102, 121, 122] Administration of chenodeoxycholic acid to young Indian women decreased the lithogenic potential of their biles.[69] By 1972 Danziger announced the successful dissolution of cholesterol gallstones by oral chenodeoxycholic acid.[123] Gallstone recurrence after discontinuation of therapy has been reported.[124] Chenodeoxycholic acid does not necessarily exert its effects by expanding the bile acid pool, but inhibits hepatic cholesterogenesis and so reduces biliary cholesterol concentrations.[110] There are a few good reviews of chenodeoxycholic acid therapy.[125, 126] Phenobarbitol has been investigated on the basis of its ability to induce liver enzymes and its potential to increase bile acid synthesis. However, results have not been encouraging.[126] Since lecithin concentration is low in gallstone patients, phospholipid feeding has been attempted.[127] It is unlikely that this form of treatment will be successful because the enterohepatic circulation of lecithin is insignificant.[96] The use of fibre in preventing and dissolving gallstones holds much promise for the future.[126]

OBJECTIVES

The objectives of this study are as follows:

1. To confirm in a Canadian population that normal bile can be separated from abnormal bile on the basis of cholesterol saturation.
2. To establish how long a lithogenic bile is secreted after cholecystectomy, and to elucidate the possible role of the gallbladder in the production of a lithogenic bile.

MATERIALS AND METHODS

Bile samples were obtained from fasting patients at the University of Alberta Hospital. Patients were selected from the operating schedule on the basis of presence or absence of biliary tract pathology, subjects with functioning gallbladders or previous cholecystectomy were considered. Subjects with elevated bilirubins, persistently abnormal liver enzyme levels or infected biles were excluded. Samples were obtained by needle aspiration of the gallbladder, needle or T-tube aspiration of the common duct, or duodenal aspiration at the time of surgery. Gallbladders were aspirated as fully as possible to ensure representative sampling.[128] Only afebrile patients with normal white blood counts were considered, and as a further check, aerobic and anaerobic cultures were taken at operation to prevent using an infected gallbladder bile for analysis.

On the basis of present or previous biliary tract pathology (as obtained from medical records) patients fell into four groups: normal biliary tract, gallstones present, previous cholecystectomy with, and without gallstones. Group I (normal biliary tract) consisted of three males and one female with an age range of 24-79, and an average age of 52 years. Cholecystectomy was performed in all cases for suspected cholecystitis, or involvement of the gallbladder in adhesions from adjacent pathology. In no cases was there any

evidence of calculi. Samples were obtained by gallbladder aspiration (Table I). Group II (gallstones present) consisted of 10 females and five males with an age range of 29-68 and an average age of 43 years. The reason for surgery was cholelithiasis. No patients had evidence of biliary tract infection. A few patients had elevations of liver enzymes but these were transient. All gallbladders were found to contain gallstones, and these were subsequently analyzed. Samples were obtained by gallbladder aspiration (Table II).

Group III (previous cholecystectomy with stones) consisted of five females and three males with an age range of 31-71, and an average of 48 years. Surgery was performed for pancreatitis, peptic ulcer, or common duct pathology (stricture and stone). Again, a few subjects had transiently elevated liver enzyme levels. Samples were obtained directly from the common duct (needle or T-tube), or from the duodenum if a clean pool of bile was encountered (Table III). Medical records confirmed the presence of calculi at the time of cholecystectomy.

Group IV (previous cholecystectomy without stones) consisted of two males and one female with an age range of 30-70, with an average of 52 years. Surgery was performed for recurrent abdominal pain, recurrent peptic ulcer, and penetrating duodenal ulcer. Samples were obtained by common duct or duodenal aspiration. Medical records confirmed that no calculi had been found at the time of cholecystectomy (Table IV).

After obtaining a sample, bile was taken immediately to

the laboratory and examined for cholesterol crystals under a polarizing microscope. Specimens were then mixed and extracted at once or stored on ice until convenient to extract (never exceeding one hour). Following extraction the specimens could be refrigerated until analysis could be completed. Stones were collected whenever possible, and allowed to dry in air prior to analysis. Total solids were determined by comparing dry weight to wet weight of specimens. Samples below 3% or above 25% total solids were excluded as recommended by Small.[41] All tests were done in duplicate, lack of agreement of greater than 4% would result in a repeat analysis. No repeats were necessary in this series however.

Phospholipids were determined by the method of Sunderman and Sunderman.[129] Extraction was accomplished as follows:

1. 1 ml specimen was added drop by drop to 22 ml $CHCl_3$ - CH_3OH (2:1) on the vortex and left to stand in excess of five minutes.
2. The tube was stoppered, shaken for 30 seconds, brought to 25 ml by adding more $CHCl_3$ - CH_3OH, and left to stand for an additional five minutes.
3. 5 ml dilute H_2SO_4 (1 ml concentrated H_2SO_4 to 2 liters deionized water) was added, the tube inverted 10 times, and left standing for 10 minutes.
4. Centrifugation at 2,000 RPM for 15 minutes separated the lower chloroform phase containing the lipids.

Phosphorus was then liberated from the lipids and reacted

with acid molybdate solution to form phosphomolybdic acid which was reduced by aminonapthol-sulfonic acid to yield a blue colour by the following method:

1. 5 ml extract was evaporated to dryness, 2.5 ml of 5N H_2SO_4 added, and the mixture slow boiled.
2. After a black or brown colour change occurred, one drop of 30% H_2O_2 was added and heating continued for at least 10 minutes until the contents became colourless. If unsuccessful this step was repeated.
3. A standard was prepared by transferring 0.5 ml of phosphate standard (0.08 mg phosphorus per ml) to a digestion tube and adding 2.5 ml of 5N H_2SO_4. 2.5 ml of 5N H_2SO_4 was used as a blank. The same amount of H_2O_2 as used in step 2 was added, and the tubes boiled for 10 minutes.
4. Contents were diluted with a few ml deionized water, cooled to room temperature, and transferred to 25 ml volumetric flasks with repeated washings so the flask was half full.
5. 2.5 ml ammonium molybdate solution (2.5% w/v) and 1 ml aminonapthol-sulfonic acid reagent were added. Contents were diluted to the 25 ml mark with deionized water, mixed, and allowed to stand for five minutes.
6. Measurements of optical density were made with the Unicam S. P. 1800 spectrophotometer at 675 nm.

7. Calculations:

$$\text{mg\% phospholipid} = \frac{\text{O.D. unknown}}{\text{O.D. standard}} \times 0.04 \times 18 \times 100 \times 25$$

Millimolar concentration was found by employing the conversion factor:

1 mM phospholipid = 793 mg/l

Cholesterol was determined by the method of Abell.[129, 130, 131] Basically bile was treated with alcoholic KOH to liberate cholesterol from lipoprotein complexes and saponify cholesterol esters. Cholesterol was extracted into petroleum ether and the Liebermann-Burchard reaction was used to produce a green colour which was measured spectrophotometrically. Reagents used were:

1. 95% ethyl alcohol, redistilled.
2. Petroleum ether B.P. 68°C, reagent grade.
3. Glacial acetic acid, reagent grade.
4. Concentrated H_2SO_4, reagent grade.
5. Acetic anhydride, reagent grade, free from HCl.
6. KOH solution 33% w/w.
7. Ethanolic KOH solution, prepared immediately before use (6 ml 33% KOH to 94 ml 95% ethyl alcohol).
8. Standard cholesterol solution (100 mg cholesterol to absolute CH_3OH to make 250 ml).
9. Modified Liebermann-Burchard reagent (1 vol. concentrated H_2SO_4 to 20 vol. acetic anhydride, chilled to less than

10°C, kept cold for nine minutes, and 10 vol. glacial acetic acid added).

The procedure was:

1. Standards - 0.3 ml of 33% KOH was added to a 25 or 50 ml glass stoppered centrifuge tube containing 5 ml cholesterol standard.
2. 0.5 ml bile were transferred to centrifuge tubes and 5 ml alcoholic KOH added. Tubes were stoppered, shaken well, and placed in a water bath at 37 - 40°C. for 55 minutes.
3. After cooling to room temperature, 10 ml of petroleum ether was added and mixed well, 5 ml water added and shaken vigorously for one minute, and centrifuged for 10 minutes.
4. Test: Duplicate 4 ml aliquots of the petroleum ether layer were transferred to large dry test tubes. Standards: duplicate aliquots, 1.0, 2.0 and 3.0 ml (equivalent to 0.2, 0.4 and 0.6 mg cholesterol) were transferred to similar test tubes. All tubes were evaporated to dryness at 60°C in a water bath while blowing a gentle stream of nitrogen into them.
5. After cooling, test tubes were arranged so that one set of standards was at the beginning and the other at the end of the series, and a clean empty test tube was placed at the beginning to receive the blank.
6. 6 ml of modified Liebermann-Burchard reagent was added,

beginning with the blank, and at one minute intervals, to the samples. Tubes were stoppered, mixed using a shaker, and optical densities read at 620 mμ exactly 30 minutes after addition of the reagent.

7. Calculations:

$$\text{Optical density equivalent to 1 mg cholesterol (S)} = \frac{\text{O.D. standard}}{\text{mg cholesterol in aliquot}}$$

$$\text{mg \% cholesterol} = \frac{\text{O.D. unknown}}{S} \times \frac{10}{4} \times \frac{100}{0.5}$$

Millimolar concentration of cholesterol was calculated using the conversion factor: 1 mM = 387 mg/l.

Enzymatic determination of bile acids was carried out using the method of Engert and Turner.[132] Reagents were:

1. Buffer, 0.1 M sodium pyrophosphate.
2. Hydrazine sulphate 1.0M (3.4 ml 95% hydrazine plus 1.5 ml concentrated H_2SO_4, Q.S. to 100 ml with deionized water).
3. NAD, 6.8mM.
4. Enzyme prepared from Pseudomonas testosteroni (Sigma, Type 1).

Procedure was carried out as per Table V. All tubes were then incubated at 37°C for 60 minutes. Spectrophotometric determinations were made at 340 nm. A graph was constructed by plotting mM concentration of chenodeoxycholic acid standards against optical density and values obtained for the bile samples from their optical

densities.

Cholesterol content of gallstones was determined by drying the stone in air and grinding it to a fine powder. Using 0.5 gm of powdered stone, cholesterol was determined as outlined above and percentage cholesterol was calculated. Relative percentages of bile acids, phospholipids and cholesterol were calculated from the total of their concentrations in millimoles. Results were plotted on the triangular coordinates of Admirand and Small (Fig.1).[41] The lithogenic index of Metzger, which is the ratio of absolute percent cholesterol to the theoretical limit of cholesterol solubility at a given concentration of phospholipid and bile salts, was calculated. Values greater than 1.0 are supersaturated, values less than 1.0 are unsaturated (Fig. 2).[133]

RESULTS

Group I consisted of one female and three males with normal biliary tracts and an age range of 24 to 79 years. Bile was unsaturated in two cases, saturated in one, and supersaturated in one. Mean lithogenic index was 0.92 (S.D. $\pm$ 0.143). Molar concentrations, percent molar concentrations and lithogenic index are listed in Table VI. No crystals were seen in these specimens.

The patients undergoing cholecystectomy, Group II, consisted of ten females and five males with an age range of 24 to 70 years. With the exception of two, all biles in this group were supersaturated with cholesterol. Crystals were seen in all specimens examined. Mean lithogenic index was 1.41 (S.D. $\pm$ 0.48). Complete results, including percentage cholesterol in gallstones, are shown in Table VII.

Previous cholecystectomy for stones, Group III, consisted of five females and three males whose ages ranged from 28 to 71 years. All bile samples in this group were supersaturated with cholesterol. Mean lithogenic index was 1.31 (S.D. $\pm$ 0.106), no crystals were seen. Complete results are shown in Table VIII.

Group IV, previous cholecystectomy without stones, consisted of two males and one female with an age range of 30 to 70 years. All bile samples were unsaturated and had a mean lithogenic index of 0.52 (S.D. $\pm$ 0.077), no crystals were seen. Results are presented in Table IX.

Mean lithogenic index plus or minus one standard deviation for all groups are represented on a bar graph (Fig. 3). Groups with stones, past or present, clearly have lithogenic bile (lithogenic index greater than 1.0) when compared to groups without stones (I and IV). Comparing percentage cholesterol from groups with gallstones (III and IV), to proposed lines of cholesterol solubility shows great similarity with respect to cholesterol supersaturation (Fig. 4). When results from groups with previous cholecystectomies, with and without stones, are compared using circles drawn around points plotted on triangular coordinates, separation on the basis of cholesterol saturation is obvious (Fig. 5). That time since cholecystectomy does not influence cholesterol saturation is illustrated in Figure 6.

DISCUSSION

In this study, only bile obtained from fasting subjects was used. Metzger has shown that fasting hepatic bile is more lithogenic than bile obtained at other times.[64] During fasting, much of the bile salt pool is stored in the gallbladder. Because much of the bile salt pool is unavailable for recirculation, there will be a reduction in bile salt secretion, *de novo* synthesis being slow to increase. Consequently during fasting hepatic bile becomes more lithogenic due to a reduced bile salt return through the liver. Cholesterol secretion may be increased due to lack of inhibition caused by reduced chenodeoxycholate return.[110] This mechanism would also make fasting bile more lithogenic. Gallbladder bile taken during fasting, however, is said to be representative of average daily bile composition. At this time several hours of pooled bile secretion by the liver are available for sampling. In the same subject, hepatic and gallbladder bile composition are similar, although gallbladder bile is more concentrated and somewhat less lithogenic.[66, 67]

Bile taken from subjects with cholecystectomies in the fasting state would presumably yield a sample with a composition

representing the mean daily secretion of lecithin, bile salts, and cholesterol, since there is no gallbladder storage and consequently no interruption of the enterohepatic circulation. For these reasons it is permissible to compare fasting gallbladder bile to fasting hepatic bile from cholecystectomized subjects. The occasional duodenal aspirate from cholecystectomized patients is also a representative sample of hepatic bile as demonstrated by Vlahcevic. He obtained samples of duodenal and gallbladder bile in the same patients at the same time and found them to be similar in composition.[134]

Patients with infected bile, jaundice or grossly abnormal liver enzymes were excluded from the study since these factors can affect bile composition. Infection can result in deconjugation of bilirubin glucuronide and its precipitation with calcium, forming a nidus for cholesterol deposition.[7] Any resultant inflammation allows bile salts to pass through the gallbladder mucosa.[20, 21] Significant liver disease results in an impaired ability to synthesize many important compounds as illustrated by cirrhotics.[52] An occasional patient was excluded because information such as gallstone analysis or past medical records was lacking. Samples outside the 3 - 25% solids range were excluded because the limit of cholesterol solubility in the quaternary system of Small would not be applicable. Analysis was carried out using precise standard reference methods in duplicate. Lack of agreement between duplicates of greater than 4% resulted in a repeat test.

Several previous studies regarding the influence of cholecystectomy have accumulated in the literature - with conflicting results. For each paper which concludes that bile remains lithogenic post-operatively, there is an equally convincing paper claiming that cholecystectomy improves the cholesterol holding capacity of bile.[55, 56, 57, 114, 115, 116, 117] In fact Small has co-authored two papers with conflicting results.[55, 66] To date there is no good explanation for this difference in results. The few papers which attempt to discuss this point provide only weak comments.[55, 117]

In our study bile was obtained from anaesthetized patients following a ten hour fast. Groups were reasonably well matched (see Methods) and no patients were taking antibiotics or estrogens. Although these criteria are present on most other studies, surgical biles have been compared to non-surgical biles in some opposing papers.[55, 56] Samples were taken either at the time of initial surgery, or at a second operation long after recovery. Sampling early after operation may introduce errors since bile secretion rates in the primate are depressed for two weeks, and synthetic rates for up to six weeks, post-operatively. Studies which show bile returns to normal used samples from indwelling T-tubes from two weeks to three months post-operatively.[55, 114] By contrast, patients in this study averaged about nine years post-cholecystectomy, did not have interrupted enterohepatic circulations, and had adequate time for their biliary physiology to stabilize.

None of the previous studies have used similar methodologies

for the analysis of bile. It is in this area that the most likely reasons for conflicting results may be found. A review of papers for methodology is somewhat frustrating however, since many are lacking in detail. The two major groups who propose that bile becomes non-lithogenic post-operatively, froze their specimens prior to analysis. As previously discussed, micellar formation occurs only above a critical temperature, the Krafft point. Freezing and thawing will disrupt micellar structure and result in cholesterol precipitation from a supersaturated solution. Mixing procedures after thawing are likely inadequate to compensate for this phenomenon. In fact, the mean bile composition of gallbladder bile from cholesterol gallstone patients in Shaffer's study is non-lithogenic![55] Post-operative mean bile composition in Simmon's study is so close to the line of cholesterol solubility that a slight error could easily shift the results to one side or the other.[56] The methods of analysis and exacting care taken with samples in our experiment insured accurate results.

Comparison of patients with a normal biliary tract (Group I) and gallstones (Group II) confirms the findings of Small in a Canadian population. That is, gallbladder bile from gallstone patients is supersaturated with cholesterol when compared to normals.[14] That one of the normals had saturated, and another supersaturated bile is explained by the high probability of "normal" individuals being in a "pre-gallstone" state in a population where the incidence

of cholelithiasis is high.[69]

The usual quoted incidence of pure or mixed cholesterol stones is 85 - 90% of all stones.[4] Stone analysis revealed only one of 15 stones (Table VII) was not predominantly cholesterol in content. The probability of more than one of the eight patients who had cholecystectomy for cholelithiasis (Group III) having other than cholesterol gallstones is low; therefore it is assumed that this group had cholecystectomies for cholesterol cholelithiasis. Comparison of this group to the others shows that bile remains persistently supersaturated in gallstone patients after cholecystectomy. Three patients who had previous cholecystectomy for acalculous disease (Group IV) continue to secrete unsaturated bile, and thus confirm that cholecystectomy does not significantly influence the lithogenicity of bile.

If chenodeoxycholic acid is used to dissolve stones, therapy will require continuous lifelong administration if cholecystectomy is to be ultimately avoided, since lithogenicity persists. Gallstone recurrence after discontinuation of therapy has in fact been reported.[124] Chenodeoxycholic acid therapy may be useful in elderly or debilitated patients who are poor surgical risks. However, for the young gallstone patients, operation will be an attractive alternative to lifelong pill-taking.

This study eliminates the possibility that the gallbladder is in some way responsible for the formation of lithogenic bile. That lithogenic bile originates from the liver is known, and it has

been suggested that cholelithiasis is a liver disease.[66, 67, 70] From a review of the literature however, it seems that dietary factors are a more likely cause of the abnormality.

Cholesterol gallstone disease is primarily one of Western countries where 85% of gallstones are of the pure or mixed cholesterol variety.[4] By comparison cholesterol gallstones are a rarity in the rural African or Asian until he adopts the Western style of life.[13] It is also true that compared to the Westerner the African has an increased amount of fibre and lignin in his diet, a bulky stool and a decreased intestinal transit time. The significance of these intestinal factors in diverticular disease of the colon has been appreciated for some time while their significance in cholesterol gallstone disease is just emerging.[37]

Cholesterol solubility in bile is critically related to the relative concentrations of bile acids, lecithin and cholesterol although there is still controversy over the exact limits of cholesterol solubility *in vivo*.[41, 59, 60] It has been demonstrated that patients with cholesterol gallstones have reduced bile acid pool sizes and that such diminution precedes stone formation.[97, 98]

Cholic acid (a trihydroxy bile acid) and chenodeoxycholic acid (a dihydroxy bile acid) are synthesized in the liver, secreted in bile and subject to bacterial deconjugation and dehydroxylation in the intestine (deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid).[71, 79] With the exception of lithocholic acid, which is poorly absorbed, the bile acids enter

the portal circulation from the distal ileum and colon, return to the liver, and are re-cycled.[72] The only significant loss from the enterohepatic circulation is through the feces. Liver synthesis of bile acids is under a feedback control mechanism whereby a decreased bile salt return through the enterohepatic circulation results in an increased synthetic rate.[75] In this regard there is evidence that increased absorption of deoxycholic acid has a selective inhibitory effect on the synthesis of chenodeoxycholic acid, but not on cholic acid.[80] This selective inhibition becomes even more significant since it has been found that chenodeoxycholate suppresses liver synthesis and output of cholesterol.[110]

Colonic stasis in Western industrialized man is responsible for increased dehydroxylation of cholate and consequently increased resorption of deoxycholate throughout the day, which results in selective suppression of chenodeoxycholic acid synthesis. Increased resorption also continues at night, when ideally much of the bile acid pool is stored in the gallbladder, and is unavailable for recirculation.[64] Synthetic rates in the primate take several hours to begin to approach maximum, following acute interruption of the enterohepatic circulation.[108] Stasis therefore results in a relative depression of synthesis at a time when *de novo* production of bile acids should be approaching maximum rates. The Westerner, therefore, produces less new bile acids in a day than the African, who must compensate for his increased excretion. The constant increased suppression of synthesis from bile acids returning

through the portal system (increased recycling rate) causes the bile acid pool to shrink so that total daily stimulus for synthesis and the mean 24 hour output return to a more normal level.[106] A reduction in pool size and decrease in synthesis of chenodeoxycholate result in the production of a bile unable to hold cholesterol in solution. The high fibre African diet reduces transit time and increases excretion by binding bile acids to lignin.[86] Consequently there is less colonic resorption of bile acids, increased bile acid synthesis, particularly during sleep, decreased recycling rate, and a bile rich in chenodeoxycholate but low in cholesterol.[109, 136] The end result is cholesterol gallstones in the Western man and not in the African.

The effect of cholecystectomy is to remove an organ that in its functioning state produces intermittency in the enterohepatic circulation of bile salts by storing bile during fasting. Its removal will result in a continual flow of bile into the gut, and therefore increase recycling rate. Bile salt pool size should consequently be reduced so that 24 hour output (pool size times recycling rate) remains relatively constant.[105, 106] Several studies confirm that pool size is reduced following cholecystectomy.[57,84,112]

The resultant increased exposure of bile salts to intestinal bacteria provides an increased opportunity for deconjugation and dehydroxylation.[84] This is reflected in an observed increase in the deoxycholate pool post-cholecystectomy.[57, 84] The study by Low-Beer

in which feeding small doses of deoxycholate to volunteers caused deoxycholate to replace chenodeoxycholate without the ratio of cholate being altered, indicates that deoxycholate selectively suppresses chenodeoxycholate synthesis.[80] An increased deoxycholate pool post-cholecystectomy should therefore produce a decrease in chenodeoxycholate synthesis and pool size. This has been confirmed by two studies of pool sizes before and after cholecystectomy.[57, 84] Chenodeoxycholate in low doses has been shown to reduce hepatic output of cholesterol by a yet undetermined mechanism.[110] Cholecystectomy would result in an increased output of cholesterol by increasing deoxycholate, which suppresses chenodeoxycholate synthesis. Feeding deoxycholate has in fact been shown to increase the cholesterol content of biliary lipids.[135]

The overall result is to maintain bile in a lithogenic state. Only if deoxycholate production was reduced by bile salt sequestering agents, such as lignin, or if intestinal transit time was greatly increased, would bile become normal, post-cholecystectomy.

The gallbladder is not responsible for the formation of lithogenic bile since lithogenicity persists in gallstone patients independently of its presence or absence. Crystals were seen only in gallbladder bile however, which suggests that cholesterol precipitation requires the elements of stasis and time. This could well explain the low incidence of common duct stones when compared to the incidence of gallstones. Several experiments in animals allude to the importance of mucopolysaccharides in the structural

formation of gallstones from cholesterol crystals. Furthermore, lithogenic bile may irritate the gallbladder and be responsible for increased mucous concentration observed in the bile of gallstone patients.[26, 36, 39, 40] Thus the gallbladder is intimately involved in the formation of gallstones once the abnormality in bile has been initiated.

CONCLUSIONS

Several conclusions can be drawn from this study. Normal bile can be separated from pathological bile on the basis of supersaturation with cholesterol. From the bile analysis of eight patients who had cholecystectomies from one to 23 years previously, it is apparent that bile remains lithogenic after cholecystectomy. A review of choledochotomy charts confirms this finding. Choledocholithiasis is relatively common years after cholecystectomy and so bile must remain lithogenic or duct stones would be a rarity. Gallstone dissolution by chenodeoxycholic acid is therefore not a viable form of therapy for cholesterol gallstones unless lifelong administration is advised. Cholecystectomy will remain the preferred treatment in young patients until a more acceptable method of maintaining bile in a non-lithogenic state is found.

The role of the gallbladder in the etiology of cholesterol cholelithiasis is probably little more than to serve as a receptacle for their formation. Bile analysis from three patients who had previous cholecystectomy for acalculous disease and from patients who had previous cholecystectomy for cholelithiasis parallels the composition of bile from persons with and without gallstones. Thus,

the possibility that the gallbladder is responsible for formation of lithogenic bile is excluded. Lithogenic bile in some way irritates gallbladder mucosa. This results in secretion of mucus which traps cholesterol crystals and allows stone formation to proceed even during the flushing action of gallbladder contraction.

A review of the literature reveals two main theories as to the formation of lithogenic bile: liver disease and diet. The geographic incidence of cholesterol cholelithiasis hints that dietary factors are the most likely etiologic agents.

TABLE I

Group I Normal Biliary Tract: Clinical Data

Patient	Age (Yrs)	Sex	Serum Bilirubin (MG%)	SGOT IU/L	Serum Alk. Phos. IU/L	Sample Obtained By	Present Diagnosis
1. (P.Y.)	24	F	0.3	20	63	Aspiration of gallbladder	Acalculous cholecystitis
2. (C.H.)	71	M	0.8	31	89	" "	Hiatus hernia
3. (J.K.)	39	M	0.6	21	71	" "	Peptic ulcer
4. (J.B.)	79	M	0.9	26	65	" "	Acalculous cholecystitis

Normal values: *Bilirubin 0.2 - I.5 MG%*

SGOT I0-50 IU/L

Alk. Phos. 20-90 IU/L

TABLE II

Group II Gallstones Present: Clinical Data

Patient	Age	Sex	Serum Bilirub (MG%)	SGOT IU/L	Alk. Phos. IU/L
1.(S.H.)	41	M	0.6	33	78
2.(L.H.)	57	F	0.4	19	81
3.(R.K.)	33	F	0.4	56	41
4.(C.W.)	42	M	0.9	37	155
5.(H.T.)	61	M	0.7	17	87
6.(J.M.)	24	F	0.3	20	58
7.(J.W.)	55	F	0.4	30	213
8.(P.H.)	29	F	0.7	288	54
9.(I.B.)	68	F	0.5	31	117
10.(E.A.	65	F	0.9	111	273
11.(Z.M.)	33	F	0.3	15	48
12.(R.D.)	25	M	no jaundice		-
13.(E.S.)	34	F	0.7	27	69
14.(R.A.)	33	M	0.8	26	74
15.(S.S.)	44	F	0.7	40	65

All patients had a diagnosis of cholecystitis. All samples were gallbladder bile obtained by needle aspiration.

TABLE III

Group III Previous Cholecystectomy With Stones: Clinical Data

Patient	Age (Yrs)	Sex	Time Since Cholecystectomy (Yrs)	Serum Bilirub (MG%)	SGOT IU/L	Serum Alk. Phos. IU/L	Sample Obtained By	Present Diagnosis
1. (R.F.)	56	F	15	0.5	33	88	Aspiration of common duct	Benign stricture
2. (N.T.)	46	F	7	0.7	44	140	Aspiration of duodenum	Reflux esophagitis
3. (W.F.)	44	M	5	0.3	89	217	Aspiration of common duct	Common duct stone
4. (M.J.)	45	F	23	0.3	42	158	Aspiration of common duct	Neuroma common bile duct
5. (I.G.)	28	F	3	0.2	29	77	T-Tube in common duct	Pancreatitis
6. (J.M.)	64	M	10	0.4	47	89	Aspiration of common duct	Pancreatitis
7. (S.M.)	31	F	1.5	0.5	31	51	Aspiration of duodenum	Duodenal ulcer Hiatus hernia
8. (G.K.)	71	M	8	0.5	20	65	Gastrostomy tube	Duodenal ulcer

TABLE IV

Group IV Previous Cholecystectomy Without Stones: Clinical Data

Patient	Age (Yrs)	Sex	Time Since Cholecystectomy (Yrs)	Serum Bilirub (MG%)	SGOT IU/L	Serum Alk. Phos. IU/L	Sample Obtained By	Past Diagnosis
1.(R.H.)	30	F	4	0.5	26	76	Aspiration of common duct	Recurrent abdominal pain
2.(R.M.)	70	M	0.3	0.3	34	96	T-Tube in common duct	Penetrating duodenal ulcer into C. B. D.
3.(E.T.)	55	M	19	0.5	23	89	Aspiration of duodenum	Adhesions between duodenum and gall-bladder

TABLE V

Bile Salt Analysis Procedure: Assay

	Blank(ml)	Test(ml)	CDCA	Standards	ml
Buffer	2.0	2.0	2.0	2.0	2.0
Hydrazine	1.0	1.0	1.0	1.0	1.0
NAD	0.5	0.5	0.5	0.5	0.5
Bile	0.003	0.003	-	-	-
Enzyme	-	0.025	0.025	0.025	0.025
Boiled Enzyme	0.025	-	-	-	-
Chenodeoxycholate Acid	-	-	0.01	0.03	0.05

TABLE VI

Group I Normal Biliary Tract: Laboratory Data

Patient	Millimoles per Litre			Per Cent of Total Millimoles			Lithogenic Index
	Bile Acids	Lecithin	Cholesterol	Bile Acids	Lecithin	Cholesterol	
1. (P.Y.)	150.6	16.3	14.3	83	9	8	1.00
2. (C.H.)	210.0	94.3	27.8	63.5	28	8.5	0.81
3. (J.K.)	62.0	14.9	6.8	74	18	8	0.82
4. (J.B.)	126.0	44.9	21.2	63	23	11	1.08
MEAN				71	20	9	0.92
S.D.				8.67	8.12	1.52	0.14

TABLE VII

Group II Gallstones Present: Laboratory Data

Patient	Millimoles per Litre			Per Cent of Total Millimoles			Lithogenic Index	% Cholesterol in Stones (dry wt)
	Bile Acids	Lecithin	Cholesterol	Bile Acids	Lecithin	Cholesterol		
1. (S.H.)	125.0	44.6	15.5	69	22	9	0.92	99
2. (L.H.)	105.0	36.9	30.6	62	21	17	1.70	95
3. (R.K.)	153.0	43.6	19.8	71	20	9	0.92	93
4. (C.W.)	177.0	64.6	35.5	64	23	13	1.29	88
5. (H.T.)	71.9	33.6	12.5	63	27	10	0.97	86
6. (J.M.)	117.0	40.3	21.4	66	22	12	1.20	26
7. (J.W.)	52.0	16.5	9.5	67	21	12	1.21	81
8. (P.H.)	54.0	18.7	10.9	65	22	13	1.29	68
9. (I.B.)	31.7	10.5	5.3	67	22	11	1.10	66
10. (E.A.)	30.0	12.6	5.1	63	26	11	1.07	79
11. (Z.M.)	58.0	18.1	21.4	60	19	21	2.10	60
12. (R.D.)	29.0	4.3	5.2	75	11	14	1.46	94
13. (E.S.)	23.9	10.6	13.7	50	22	28	2.70	73
14. (R.A.)	98.0	56.0	18.0	56	33	11	1.18	78
15. (S.S.)	46.0	27.7	16.9	51	30	19	2.06	65
MEAN				63	23	14	1.41	
S.D.				6.7	5.0	5.2	0.48	

TABLE VIII

Group III Previous Cholecystectomy with Stones: Laboratory Data

Patient	Millimoles per Litre			Per Cent of Total Millimoles			Lithogenic Index
	Bile Acids	Lecithin	Cholesterol	Bile Acids	Lecithin	Cholesterol	
1. (R.F.)	44.3	15.2	6.6	67	23	10	1.00
2. (N.T.)	23.0	5.1	4.1	72	15	13	1.44
3. (W.F.)	62.0	16.2	9.5	71	18	11	1.11
4. (M.J.)	28.1	11.1	4.9	64	25	11	1.09
5. (I.G.)	42.2	11.2	6.4	70	18	12	1.22
6. (J.M.)	33.5	13.1	6.3	63	25	12	1.18
7. (S.M.)	17.5	7.1	4.0	61	25	14	1.36
8. (G.K.)	14.0	2.9	3.9	67	14	9	2.06
MEAN				67	25	11	1.31
S.D.				3.9	4.7	2.8	0.11

TABLE IX

Group IV Previous Cholecystectomy Without Stones: Laboratory Data

Patient	Millimoles per Litre			Per Cent of Total Millimoles			Lithogenic Index
	Bile Acids	Lecithin	Cholesterol	Bile Acids	Lecithin	Cholesterol	
1. (R.H.)	46.8	7.7	2.5	82	13	4.5	0.54
2. (R.M.)	58.0	12.4	3.0	79	17	4.0	0.44
3. (E.T.)	34.0	14.7	3.1	66	28	6.0	0.59
MEAN				76	19	4.8	0.52
S.D.				6.6	8.8	1.1	0.08

Fig. 1. Triangular Coordinates of Admirand and Small

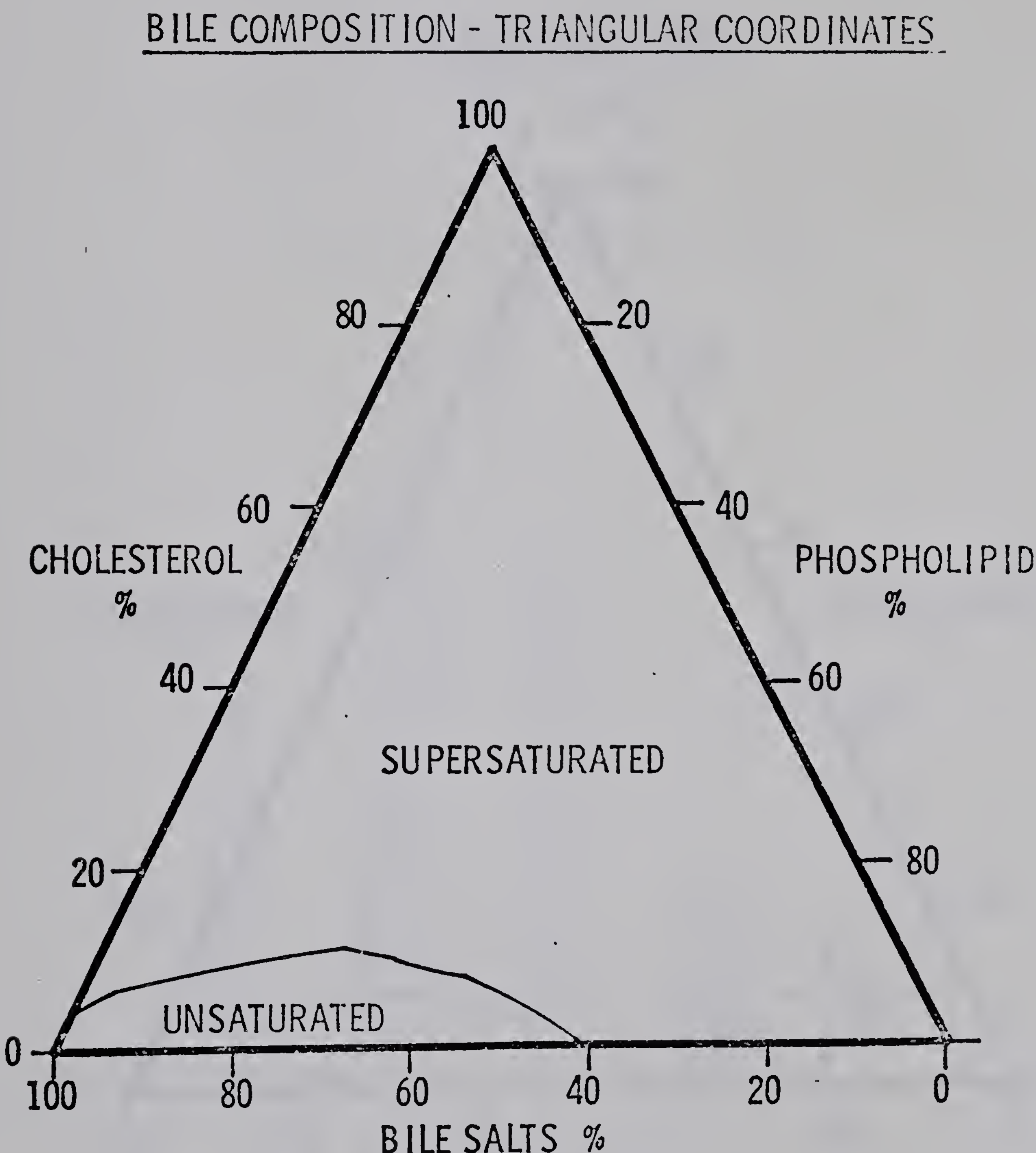


Fig. 2. Calculation of Lithogenic Index

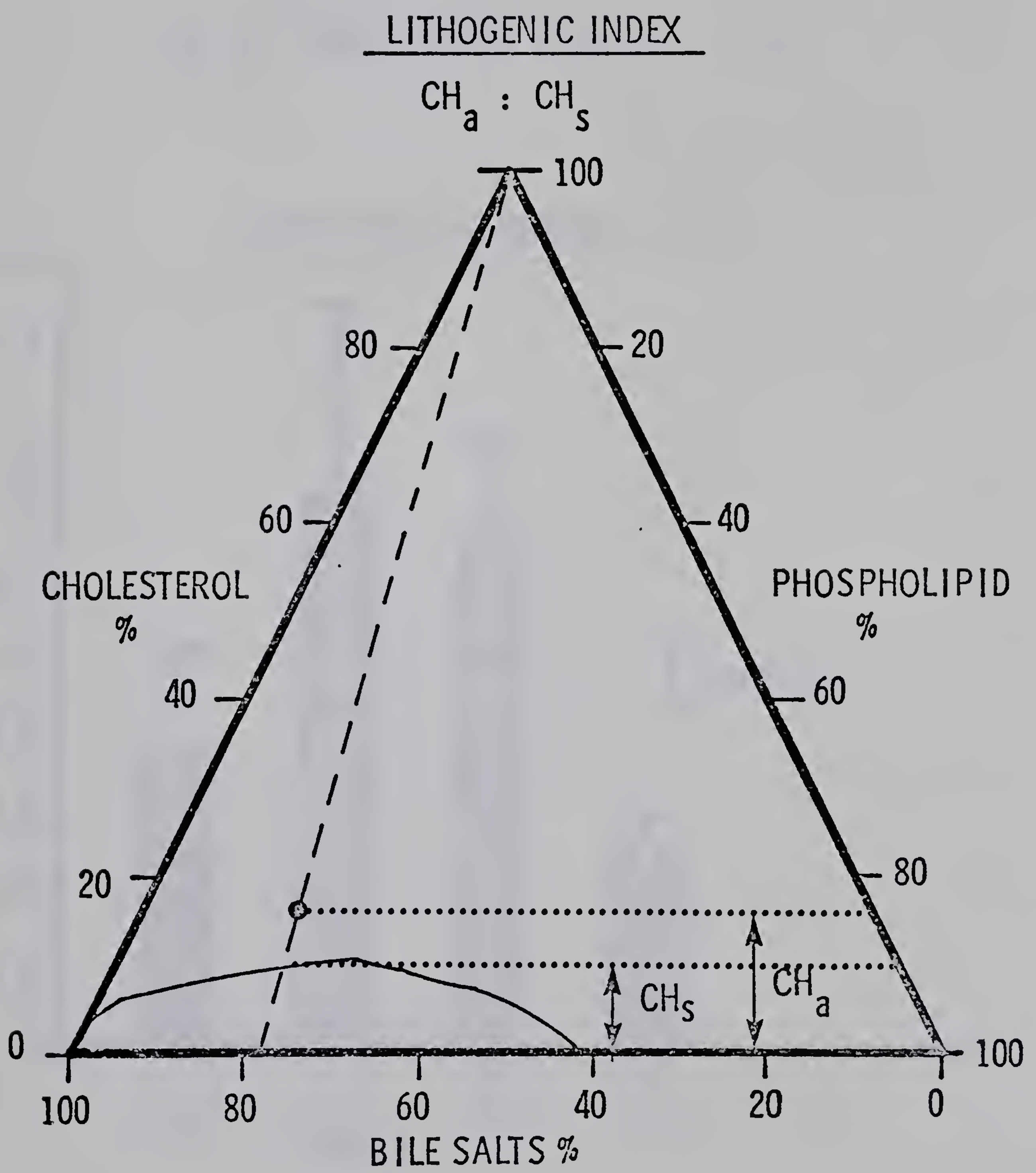


Fig. 3. Comparison of Lithogenic Index

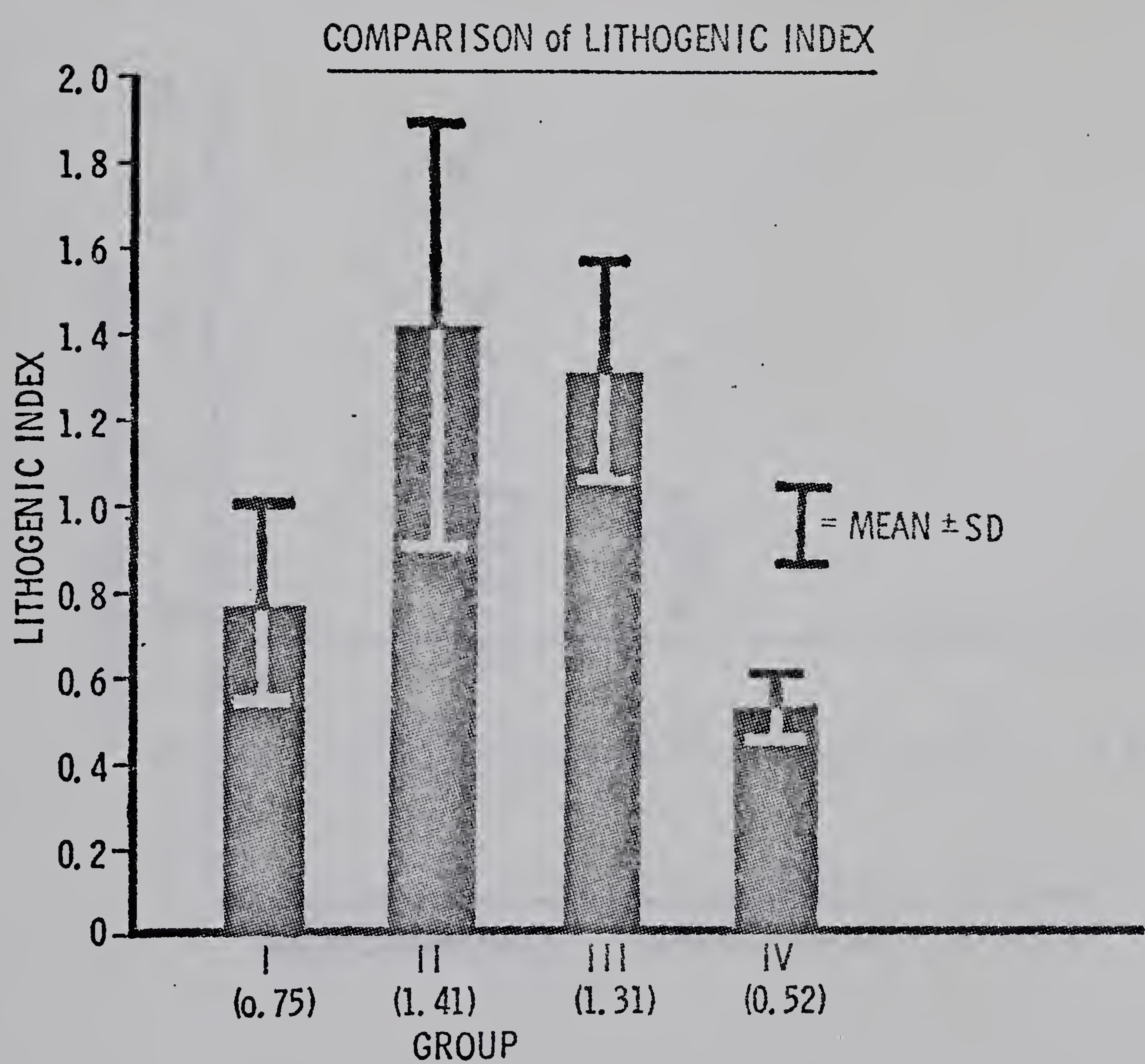


Fig. 4.

Comparison of Cholesterol Molar Percent in Groups with Gallstones Present (Cholecystectomy) and Previous Cholecystectomy with Stones. "A" and "B" Represent Lines of Cholesterol Solubility as Defined by Small and Holzbach Respectively

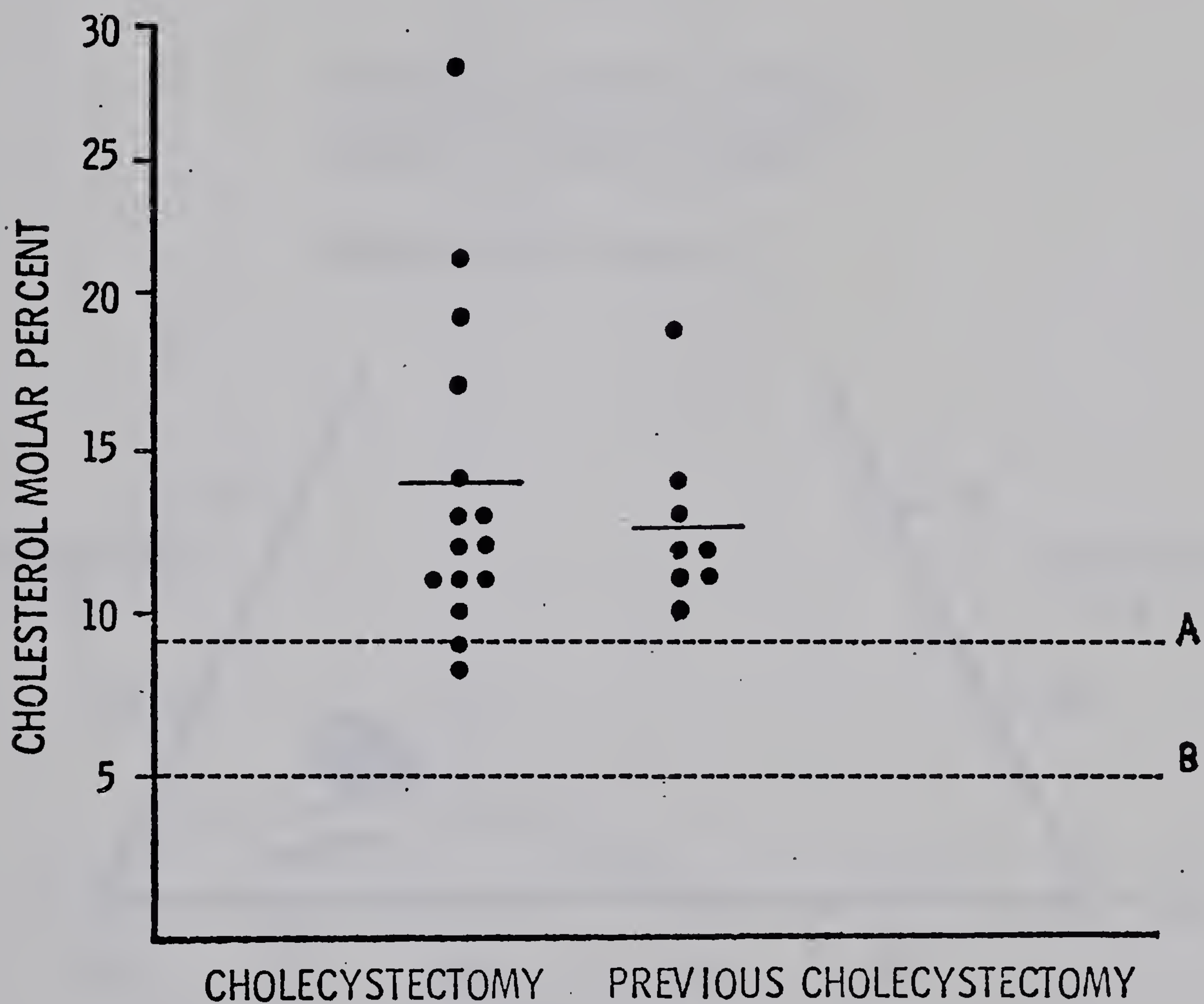
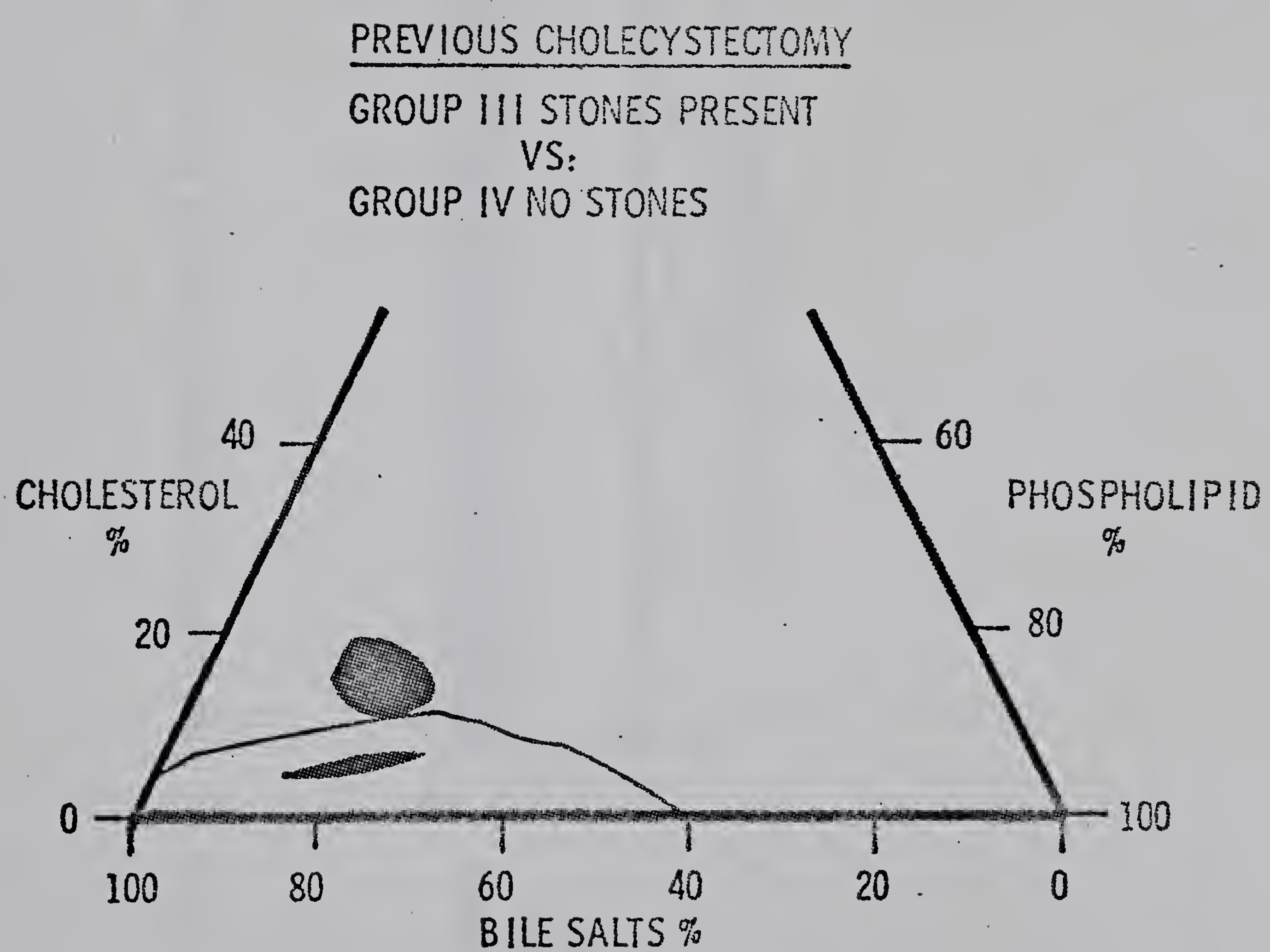


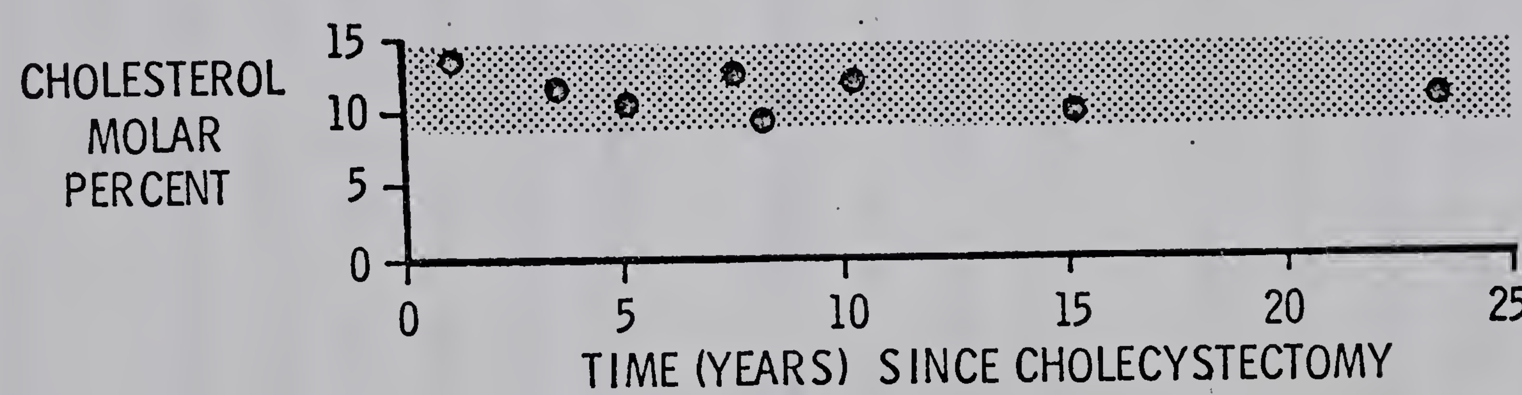
Fig. 5.



Top Circle - Group III

Bottom Circle - Group IV

Fig. 6. Effect of Time Since Cholecystectomy on Molar Percent Cholesterol



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